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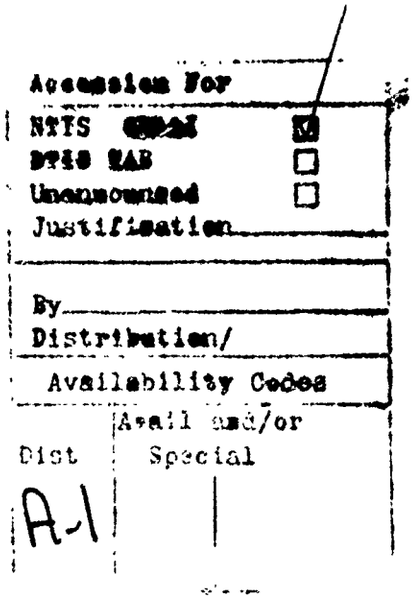

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Synthesis and opioid activity of dynorphin A(1–13) analogs substituted at positions 2 and 4

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Introduction

The dynorphin A (Dyn A) analog [Ala^2,Trp^4]Dyn A(1–13) has been reported to antagonize the activity of Dyn A(1–13) in the guinea pig ileum (GPI) assay [1]. We therefore prepared a series of [Trp^4]Dyn A(1–13) analogs containing various D- and L-amino acids at position 2 in order to examine the SAR for opioid antagonist. The analogs were also examined by fluorescence energy transfer [2,3] to see if any conformational differences between the peptides could be detected by this method.

Results and Discussion

Peptide synthesis

The peptides were prepared by SPPS on a hydroxymethylphenoxyacetic acid resin (PAC® resin, Milligen/Biosearch) using Fmoc-protected amino acids. Side chain protecting groups used were Pmc for Arg, tBu for Tyr, and Boc for Lys. Cleavage from the resin with concentrated TFA in the presence of scavengers (Reagent K) [4] yielded alkylated peptides in addition to the desired peptides. Thus, the scavenger cocktail was not able to completely suppress the alkylation of Trp in these analogs.

Pharmacological assays

Opioid receptor affinities of the pure peptides were examined in binding assays against [^{3}H]bremazocine (κ), [^{3}H]DAMGO (μ), and [^{3}H]DPDPE (δ) and their opioid activity determined in GPI assay (Table 1). Substantial differences in opioid receptor affinities and selectivity were observed for the different analogs. Only [L-Leu^2,Trp^4]Dyn A(1–13) showed selectivity for κ-receptors similar to Dyn A(1–13). [L-Ala^2,Trp^4]Dyn A(1–13) preferentially interacted with μ-receptors. In the GPI, the peptides containing D-amino acids at position 2 were much more potent agonists than the corresponding L-amino acid containing analogs. At 0.1 μM neither [L-Ala^2,Trp^4]- nor [L-Leu^2,Trp^4]Dyn A(1–13) exhibited significant antagonism in the GPI against Dyn A(1–13).

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Table 1 *Opioid activity and receptor affinity of Dyn A(1–13) analogs*

[X², Trp⁴]	GPI	K_i (nM)			K_i ratio
Dyn A(1–13)	IC_{50} (nM)	κ	μ	δ	κ/μ/d
[Ala², Trp⁴]	2310	35.4	3.52	185	10/1/53
[Asn², Trp⁴]	>1000	12.4	18.4	171	1/1.5/14
[Leu², Trp⁴]	>1000	13.4	72.2	1180	1/5.4/88
[D-Ala², Trp⁴]	9.2	5.3	0.14	8.0	38/1/57
[D-Asn², Trp⁴]	0.828	0.172	0.035	1.6	4.9/1/46
[D-Leu², Trp⁴]	29.8	25.6	0.249	249.3	103/1/1000
Dyn A-(1-13)	0.246	3.92	0.193	2.52	1/5.7/74

Fluorescence energy transfer

Fluorescence energy transfer between Tyr¹ and Trp⁴ was used to study possible conformational differences between the analogs. Fluorescence measurements were made with 10 μM peptide in 5 mM Tris buffer (pH 7.5). In a preliminary study, significant differences between the analogs could not be detected by this method.

The amino acid at position 2 influenced the opioid activity, receptor affinity and receptor selectivity of the 2,4-disubstituted dynorphin analogs. The D-amino acid-containing analogs showed significant agonist activity in the GPI and preferentially interacted with μ receptors. Derivatives containing an L-amino acid at position 2 showed little agonist activity in the GPI, while retaining opioid receptor affinity. Antagonist activity was not observed for the L-amino acid analogs tested at 0.1 μM in the GPI.

Acknowledgements

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